Kangas, H., Suurnäkki, A. and Kleen, M. (2007). Modification of the surface chemistry of TMP with enzymes. Nordic Pulp and Paper Research Journal 22 (4): 415-423.

Modification of the surface chemistry of TMP with enzymes

Heli Kangas, KCL, Espoo, Finland, **Anna Suurnäkki,** VTT, Espoo, Finland, **Marjatta Kleen,** KCL, Espoo, Finland

KEYWORDS: Enzymatic treatment, ESCA, Extractives, Fiber, Fibril, Flake, Lignin, Polysaccharide, Surface chemistry, Thermomechanical pulp, TMP, ToF-SIMS

SUMMARY: The surface chemistry of thermomechanical pulp (TMP) was modified with specific enzymes and the effect of extensive enzymatic treatments was studied with surface-sensitive spectroscopic methods (ESCA and ToF-SIMS) and by contact angle measurements. Fibers and different types of fines, i.e. fibrils and flakes, were isolated from the enzymatically treated pulps and analyzed separately. Enzymatic treatment had a pronounced effect on surface extractives, especially those found on the surface of fibrils. All the enzymatic treatments decreased the surface coverage of extractives on fibrils, but ToF-SIMS results indicate that only lipase and laccase treatments selectively removed extractives from the surfaces of fibrils and that other treatments only made the film of extractives thinner. Laccase treatment also reduced the content of guaiacylic lignin units on the surface of fibers, fibrils and flakes. All the treatments studied here lowered the contact angle of water on pulp handsheets, probably due to removal of extractives from the surface.

ESCA and ToF-SIMS gave complementary information about the surface chemistry of pulp fibers and fines. ToF-SIMS was found to be suitable for studying chemical modifications on the surface of mechanical pulp components. When these two analytical techniques are combined, it must be remembered that their analysis depth is different, that ESCA results are dependent on proper extraction, and that ToF-SIMS is not a quantitative analysis method for organics.

ADDRESSES OF THE AUTHORS: Heli Kangas (heli.kangas@kcl.fi) and **Marjatta Kleen** (marjatta.kleen@kcl.fi): KCL, P.O. Box 70, FI-02151 Espoo, Finland.
Anna Suurnäkki (anna.suurnakki@vtt.fi): VTT, P.O. Box 1000, FI-02044 VTT, Espoo, Finland.
Corresponding author: Heli Kangas

The surface chemical properties of mechanical pulps are of great importance during both papermaking and finishing operations. Surface chemistry may influence fiber bonding and charge, consumption of papermaking chemicals, adsorption of liquids onto paper, adhesion of paper to roll surface, and spreading of printing ink onto paper.

The surface chemical properties of mechanical pulps have been studied with surface-sensitive analysis techniques, such as Electron Spectroscopy for Chemical Analysis (ESCA, also called X-ray Photoelectron Spectroscopy or XPS) (Dorris and Gray 1978; Koljonen et al. 1997; Börås and Gatenholm 1999; Luukko et al. 1999; Westermark 1999; Kleen et al. 2003; Mosbye 2003; Kangas and Kleen 2004) and Time-of-flight Secondary Ion Mass Spectrometry (ToF-SIMS) (Kleen et al. 2003; Kangas and Kleen 2004; Kangas et al. 2004; Kokkonen et al. 2004; Fardim and Holmbom 2005). ESCA provides information about the elements on the surface and their bonding down to a depth of 5-10 nm. In the case of pulp samples, the carbon and oxygen atoms as well as the chemical shifts of carbon are usually measured and the surface area covered by lignin and extractives is calculated. ESCA is considered to be a quantitative or at least semi-quantitative surface analysis method. ToF-SIMS is a surface analysis technique relatively new to the paper industry, providing structural information about the surface components in the form of mass spectra. ToF-SIMS is a very surface-sensitive method, giving information from the first molecular layer (~1 nm). However, ToF-SIMS provides only qualitative or sometimes semi-quantitative information about organic material in wood fibers. In addition to spectroscopic methods, contact angle measurements may also be used to study the surface chemistry of pulps, e.g. by determining the hydrophilicity and hydrophobicity of a surface. This method is also very surface sensitive.

Due to their specificity and macromolecular size, enzymes are excellent tools for the modification and analysis of surface chemical properties of mechanical pulps (Mustranta et al. 2000). In spruce TMP, the carbohydrates, cellulose, galactoglucomannan, xylan, and pectin (polygalacturonic acid) located on the accessible pulp surfaces (outer surface of fibers and fines) can be hydrolyzed and consequently removed from pulp by cellulase, mannanase, xylanase, and pectinase treatments, respectively. The degree of carbohydrate solubilization can be adjusted by the selection of enzyme type, enzyme dosage and treatment conditions. In addition to modifying the surface of mechanical pulps, enzymes can react with dissolved and colloidal substances (DCS) in the water phase (Thornton 1994; Buchert et al. 2002). Lignin, resin and fatty acids and esterified extractives are the main constituents of the colloidal particles, while lignans and neutral polysaccharides are mostly dissolved (Ekman et al. 1990; Zhang et al. 2000).

In mechanical pulps, mannanase treatment has been found to result in destabilization and finally in attachment of colloidal resin to pulp (Kantelinen et al. 1995; Buchert et al. 1999). Pectinase treatment has been used for depolymerization of pectin (polygalacturonic acid) in anionic trash of peroxide-bleached mechanical pulps and thus to reduce the need for cationic chemicals in pulp processing (Thornton 1994; Thornton et al. 1996; Reid and Ricard 2000). Pectinases have not been found to act on fiber-bound pectin (Mustranta et al. 2001), although pretreatment of wood chips with commercial pectinase has been found to reduce the refining energy and result in a more selective fiber separation (Peng et al. 2003), indicating that modification of the compound middle lamella must have taken place during the treatment. Lipases have been used to hydrolyze triglyceri-

des released during mechanical pulping, resulting in a decrease in the content of triglycerides and an increase in free fatty acids in the pulp filtrate (Hata et al. 1996). The adsorption of fatty acids onto fibers may even increase the overall concentration of extractives in pulp sheets after lipase treatment (Mustranta et al. 2001). Surface lignin can be oxidized by phenoloxidizing enzymes such as laccase. Laccase treatment of TMP has been reported to polymerize dissolved lignans from the suspension back onto the pulp surface (Buchert et al. 1999). A reduction in the amount of hydrophilic and lipophilic extractives has also been observed after laccase treatment (Buchert et al. 1999; Zhang et al. 2000). Laccase is able to attack fatty acids with several double bonds and resin acids containing conjugated structures (Karlsson et al. 2001).

In the present work, spruce thermomechanical pulp (TMP) was treated with mannanase, xylanase, pectinase, lipase and laccase, the targets being the most common fiber wall components and dissolved and colloidal substances. Protease was also used to degrade any protein that might be present in minor quantities on the TMP fiber surface. The effect of enzyme treatment on the surface chemistry of TMP was studied with surface-sensitive analytical methods such as contact angle measurements, ESCA and ToF-SIMS. The surface chemistry of different pulp constituents, i.e. fibers, fibrils and flakes, was also studied. The goal was to gain information about how TMP can be treated with different enzymes and how the treatments change the surface chemistry of pulp components. Another goal was to evaluate the applicability of ToF-SIMS for the study of chemical surface modifications.

Materials and Methods

Pulp, enzymes and treatments

Unbleached spruce (Picea abies) thermomechanical pulp (TMP) was taken in March from a Finnish pulp mill after the second refiner. The consistency of the pulp was 55%. The Canadian standard freeness (CSF) of the pulp was 124 ml and the fines content (Bauer McNett) 25%. The content of acetone-soluble matter of the pulp was 1.6%. The pulp was stored in a freezer (-23°C) until needed.

The pulp was treated with six different enzymes: mannanase, xylanase, lipase, laccase, pectinase, and protease. Experimental mannanase preparation and xylanase (Ecopulp X-200) were obtained from Röhm Enzyme, Finland, while lipase (Resinase A 2X) and pectinase (Pectinex 3XL) were purchased from Novozyme, Denmark. Laccase was a partially purified culture filtrate from *Trametes hirsuta* (Poppius-Levlin et al. 1999). Protease was a Bromelain concentrate from Genencor. The enzyme dosage used in laccase treatment was 500 nkat/g pulp [calculated as oven dried (o.d.)], 1000 nkat/g pulp (o.d) in treatments with hydrolytic enzymes and 10 mg/g pulp (o.d) in the protease treatment. In the case of pectinase, the enzyme was dosed according to polygalacturonidase (PG) activity. Laccase activity was determined using ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) as substrate (Niku-Paavola et al. 1988).

Prior to enzyme treatments, the pulp was stored in a freezer. The defrozen pulp was homogenized using a pilot homogenizer. The homogenized pulp was diluted to 3% consistency with ion exchanged water and mixed with laboratory paddle mixer. The pH of the suspension was adjusted to pH 5. Enzyme treatments were then performed at 45°C for 24 h. The treatment conditions were not optimal for every enzyme, but they were kept constant in order to make the results comparable. During laccase treatment, oxygen was bubbled through the reaction vessel. After the treatment, excess water was removed from the pulp by filtration in a Büchner funnel. The filtrate was recirculated through the pulp pad in order to retain as much as possible of the fines. A reference treatment was carried out as explained above but without enzyme addition. The treated pulps were stored in a freezer until needed. The filtrates from the pulp samples were cooked immediately to inactivate the enzyme activities, after which they were frozen for further analysis.

Filtrate analysis

The pulp filtrates were analyzed for the components dissolved during enzymatic treatment. The content of neutral and acidic monosaccharides was determined after xylanase, mannanase and pectinase treatments. Dissolved oligosaccharides were hydrolyzed with acid to monosaccharides followed by HPLC analysis. The acidic monosaccharides were analyzed by HPLC after secondary enzymatic hydrolysis of the filtrates (Buchert et al. 1993). The amount of the dissolved mannan was calculated assuming the ratio acetyl:galactose:glucose:mannose to be 1:1:1:3 in mannan dissolved in the reference treatment and 1:0.5:1:4 in mannan released to filtrates in the enzymatic treatments. In addition, in calculation of mannan in the enzymatic filtrates, the monosaccharide contents in the reference filtrate were first subtracted from the monosaccharide contents in the enzymatic filtrates. The ratio arabinose:4-O-methylglucuronic acid:xylose was assumed to be 1.3:2:10 in xylan (Sjöström 1993). The results were converted to polysaccharides using factors 0.9 and 0.88, respectively (Sjöström 1993). Galacturonic acid was converted to polygalacturonic acid (pectin) using a factor 0.9. Dissolved wood extractives in the pulp filtrates were analyzed after lipase and laccase treatments by GC (Örså and Holmbom 1994). After protease treatment, the dissolved protein was determined by nitrogen analysis according to the EBC 4.9.1 method. The content of dissolved lignin was analyzed spectrometrically by measuring the UV at 280 nm from the laccase filtrate. The filtrate from the reference pulp was subjected to the same analyses.

Isolation of fines from pulps

Fibers and fines were isolated using a Dynamic Drainage Jar (DDJ) equipped with a 200 mesh (76 µm) wire and propeller stirring. The fines fraction passed through the

wire while the fibers were retained on the screen. The different types of fines, i.e. fibrils and flakes, were further separated from each other using sedimentation. The total fines fraction was first allowed to sediment for two days, after which the supernatant enriched in fibrils was collected. Excess water was removed from the fibrils by centrifugation (5500 rpm, 15 min). The sediment containing flakes was again diluted to 10 liters with distilled water and allowed to settle overnight, after which the clear supernatant was removed and discarded. The sediment was washed three times in a similar way.

The compositions of enriched fibrils and flakes fractions were studied by the image analysis method developed by Luukko et al. (1997) and further improved by Metso Paper. This method classifies fines into fibrillar and non-fibrillar material and can also identify ray cells. The isolation of different types of fines into separate fractions was considered successful, since the content of fibrillar material in the fraction of fibrils increased from about 40% to about 65% and the content of non-fibrillar material in the fraction of flakes increased to about 85%, of which around 17% were identified as ray cells.

Sheet preparation

Small sheets were prepared from the different pulp fractions for spectroscopic analysis. The sheets were made in a glass funnel on a 20 µm nylon screen, dried between blotters and stored in a freezer. A portion of each sheet was extracted in a Soxhlet apparatus with 120 ml of acetone for 4 h. The sample preparation prior to surface spectroscopy has to be done with great care so that it does not influence the validity of the results. In the sheet preparation prior to surface spectroscopy, there is a risk that the extractives present in the water phase will be retained in the sheet during filtration. However, excess water was removed from the fibrils by centrifugation, as recommended by Örså and Holmbom (1994). In our previous work, we studied the effect of the isolation procedure on the surface chemistry of fibrils (Kangas and Kleen 2004). It was found out that most of the extractives were removed with the supernatant after centrifugation and that the centrifugation speed had no notably effect on the content of surface extractives on fibrils.

For contact angle measurements, 60 g/m^2 laboratory sheets were prepared according to standard ISO 5269-1 (2000). Prior to sheet preparation, the pulps were hot disintegrated according to standard ISO 5263 at 85°C for 10 min (1995). The sheets used for contact angle measurements were calendered on one side with a GRADEK laboratory calender using one nip. The linear load in calendering was adjusted to obtain the same roughness levels on the sheets and was 160 kN/m for protease and reference treated samples and 130 kN/m for the other samples. More water was used in the preparation of handsheets than of the small sheets, so their surface chemistries may differ. However, this fact gave the opportunity to study the effect of washing on the surface chemistry of enzymatically treated pulps.

ESCA analysis

The ESCA analysis was performed with a Kratos Analytical AXIS 165 high-resolution electron spectrometer at the Center for Chemical Analysis, Helsinki University of Technology, Finland as described by Kangas and Kleen (2004). Three spots were analyzed on each sheet. The surface coverage (atom-%) of lignin and extractives was calculated from the averaged C-C percentages in high-resolution C 1s spectra (Kleen et al. 2002). The surface coverage of polysaccharides was calculated as the difference (100 – coverage of lignin – coverage of extractives)%.

ToF-SIMS analysis

Small sheets that had not been extracted were subjected to ToF-SIMS analysis. The instrument used was a PHI TRIFT II from Physical Electronics, located at Top Analytica Ltd., Turku, Finland and the measurements were made as described by Kangas and Kleen (2004). Three replicate analyses were performed from each sample. Peak identification in ToF-SIMS spectra was based on model compound analysis as described for extractives by Kangas and Kleen (2004). Peaks originating from guaiacylic units of lignin, at 137 and 151 m/z, were identified based on the analysis of milled wood lignin (MWL) from spruce wood (Kleen 2000) and spruce TMP. The peaks originating from pentosaccharides, mainly xylan, at 115 and 133 m/z and from hexosaccharides, i.e. cellulose, mannan and galactan, at 127 and 145 m/z were identified based on the analysis of numerous model compounds. The peaks identified in the positive ToF-SIMS spectrum were integrated and normalized to the total intensity of the spectrum. An average value for each peak was calculated from the three analyses. Standard deviations were usually well below 10%. The peaks were divided into six groups based on their origin, and the intensities of peaks in each group were added together. This procedure provides a semi-quantitative method to study the effect of enzymatic treatment on the surface chemistry of pulp components.

Contact angle measurements

The advancing contact angle of water was measured with a FIBRO 1000 Dynamic Absorption Tester (DAT). The contact angles of water on the calendered sheet surface were determined as a function of time from 0.02 s up to 2-5 s depending on the sample. The drop volume, base and height were also monitored. Contact angles were calculated from the height and volume of the drop, assuming that its shape was spherical. Ten parallel analyses were made for each sample.

Results and Discussion

Compounds dissolved in enzymatic treatments

The original, unbleached TMP contained (by weight) 1.6% extractives, 27.8% lignin, 34.2% cellulose, 12.3% (galacto)glucomannan, 6.8% xylan and 2.6% pectin (polygalacturonic acid) as reported previously (Kangas

and Kleen 2004). Because of the numerous steps in the analytical procedure, the total weight percentage does not add up to 100%. The dissolved amounts of galactoglucomannan, xylan and polygalacturonic acid were compared to their contents in the original pulp.

The carbohydrate compositions reported as monosaccharides analyzed after reference, xylanase, mannanase and pectinase treatments are shown in *Table 1*. The monosaccharide contents were converted to polysaccharides and pectin (polygalacturonic acid) (*Table 2*). The analysis of pulp filtrates revealed that during reference treatment without enzyme addition, 1.1% of the pulp dry weight was dissolved, mainly as galactoglucomannan, which is known to be water-soluble (Sjöström 1993; Kleen and Lindström 1994) (*Table 2*). In the mannanase treatment, 3.4% of the pulp dry weight was dissolved, which corresponded to over 20% of the initial (galacto)glucomannan content of the pulp. This means that mannanase vas able to dissolve about 13% of the pulp (galacto)glucomannan. In the xylanase treatment, 2.1% of the pulp dry weight was dissolved, corresponding to 5.5% of the initial xylan. Mannanase and xylanase were semi-commercial and commercial preparations, respectively, and had side activities. Mannanase had some xylanase activity and xylanase had some mannanase activity, as seen in *Table 2*. The higher degree of solubilization in the mannanase treatment is in agreement with the previously reported work by Mustranta et al. (2000) and indicates more accessible location of glucomannan as compared with xylan on TMP surfaces. In the pectinase treatment, 1.6% of the pulp dry weight was dissolved, corresponding to only 1% of the original pectin (polygalacturonic acid) content.

The compositions of dissolved extractives in the pulp filtrates after reference, lipase and laccase treatments are shown in *Table 3*. In the lipase treatment, the content of triglycerides decreased and that of free fatty acids increased, indicating the hydrolysis of triglycerides to fatty acids. A similar finding has been reported by (Hata et al. 1996). Laccase treatment reduced the content of resin acids, sitosterol, steryl esters and triglycerides in the pulp filtrate, indicating oxidation of these compounds in the laccase reaction and their degradation or modification. A similar result has been reported by Buchert et al. (1999). The content of free fatty acids in the filtrate increased, possibly because free fatty acids were released from steryl esters and triglycerides. The content of dissolved lignans also decreased during laccase treatment, indicating the oxidation of lignans and the adsorption of polymerized lignans onto the fiber surface (Buchert et al. 1999). Redeposition of oxidized lignans was also observed as a darker color of the laccase-treated pulp.

Table 1. The carbohydrate composition of the pulp filtrates after reference and enzymatic treatments (mg/g o.d. pulp). Glu = glucose, Man = mannose, Gal = galactose, Xyl = xylose, Ara = arabinose, MeGluA = methylglucuronic acid and MeGalA = methylgalacturonic acid.

	Total	Dissolved carbohydrates as monosaccharides (mg/g)						
	mg/g	Glu	Man	Gal	Xyl	Ara	MeGlcA	MeGalA
Reference	11.3	3.7	5.8	1.5	0	0.3	0	0
Xylanase	21.5	5.0	10.3	1.9	3.2	0.6	0.2	0
Mannanase	34.4	9.7	18.6	2.2	2.5	0.6	0.4	0.1
Pectinase	16.1	3.7	8.0	1.8	1.6	0.3	0.2	0.3

Table 2. Solubilized material, % of original.

	Total material	Mannan	Xylan	Pectin (polygalacturonic acid)
Reference	1.1	6.6	0	0
Xylanase	2.1	4.7*	5.5	0
Mannanase	3.4	13.3*	4.3	0.3
Pectinase	1.6	2.3*	2.8	1.0

* calculated from monosaccharide contents after subtraction of the monosaccharides in the reference filtrate

Table 3. The composition of dissolved wood extractives (mg/l) in the pulp filtrates after enzymatic treatments. FFA = free fatty acids, RA = resin acids, LIGN =lignans, ST = sitosterol, SE = steryl esters and TG = triglycerides.

	FFA	RA	ST	SE	TG	Total lipophilics	LIGN
Reference	5.0	6.6	2.6	9.0	14.9	38.1	41.2
Lipase	17.1	8.9	2.5	9.4	1.3	39.2	41.3
Laccase	23.1	<0.5	0.8	4.3	3.7	32.4	12.7

The content of dissolved nitrogen in the pulp filtrate after protease treatment was 36 mg/l, compared with 33 mg/l in the reference treated pulp filtrate (results not shown). The difference fits into the experimental error and indicates that the protein content of the pulp was little affected by the protease treatment. This could be due to the inaccessibility of the fiber protein. The measured absorbance of the laccase filtrate at 280 nm was increased to 6.5 from the value of 3.2 in the reference filtrate, which would normally indicate increased lignin content in the laccase filtrate. However, proteins (i.e. enzymes) also have an absorption maximum at 280 nm and this was probably the main reason for the increased absorbance value.

Surface coverage of extractives, lignin and polysaccharides by ESCA

Fibers

Previously, we have found that the outer surface of fibers from second-stage TMP consists mostly of S2-layer, with some remnants from the outer layers (Kangas et al. 2004). According to ESCA analysis, around 50% of the reference treated fiber surface area was covered with polysaccharides and around 35% with lignin (*Fig 1A*). The differences in polysaccharide coverage between reference fibers and fibers separated from the enzymatically treated pulp were small and within experimental error.

Mannanase and xylanase treatments both increased the surface coverage of lignin, probably due to removal of glucomannan and xylan from the fiber surface followed by the exposure of lignin. Pectinase treatment increased the surface coverage of extractives slightly, possibly due to adsorption of extractives from the water phase. The changes on the fiber surface induced by lipase, laccase and protease treatments were within the experimental error.

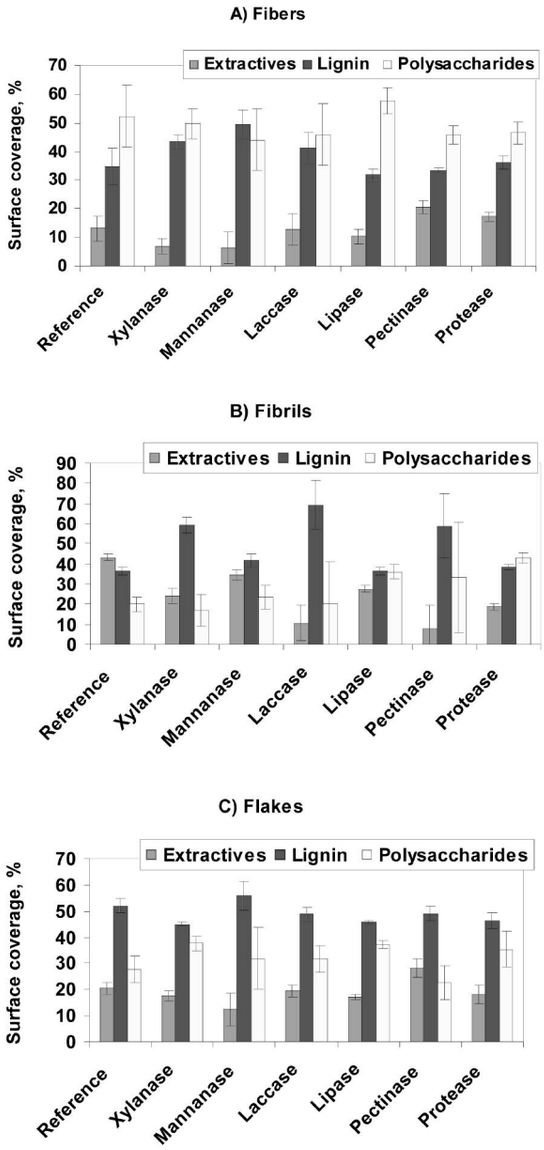

Fig 1. The surface composition (% area) of enzymatically treated A) fibers, B) fibrils and C) flakes measured by ESCA.

Fibrils

After reference treatment, fibrillar fines had a high surface area covered by extractives (*Fig 1B*). The surface extractives probably originated from the dissolved and colloidal substances which were present in the mill white water after refining and which had adsorbed onto the fibril surfaces due to their large specific surface area (Wood et al. 1991).

All the enzymatic treatments lowered the surface coverage of extractives on fibrils (*Fig 1B*) in the order pectinase > laccase > protease > xylanase > lipase > mannanase. Laccase is known to have specific reactions with triglycerides, fatty acids and resin acids (Karlsson et al. 2001). With the exception of fatty acids, the content of extractives in the pulp filtrate also decreased during laccase treatment (*Table 3*), indicating that laccase was also reactive towards dispersed and colloidal extractives. The decrease in the surface coverage of extractives during lipase treatment indicated that lipase effectively hydrolyzed triglycerides present on the surfaces of fibrils, besides those in the water phase. The free fatty acids formed were dispersed in the water phase and did not adsorb onto the fibril surfaces (*Table 3*). Previously, lipase treatment was found to decrease the surface coverage of extractives in mechanical pulp handsheets, even though the overall content of lipophilic extractives remained the same (Buchert et al. 1999), indicating that the extractives removed had formed a thin layer on the surface of handsheets.

Both mannanase and xylanase treatments have been found to lead to destabilization of colloidal extractives (Kantelinen et al. 1995), to the adsorption of extractives onto pulp, and to increased surface coverage of extractives. The decrease in the surface coverage of extractives on fibrils could be due to the fact that the removal of glucomannan and xylan from the surfaces has also led to partial removal of extractives, for example, if the extractives were in some way aggregated with the carbohydrates. Another explanation, which may also explain the effect of pectinase and protease treatment, is the increased dispersability of extractives during the long treatments. If the extractives are attached loosely to the fibrils, they may be released by the action of enzymes on the fibrils.

The decrease in the coverage of extractives on fibrils was accompanied by an increase in the surface coverage of polysaccharides during lipase and protease treatments and by an increase in the surface coverage of lignin during xylanase, laccase and pectinase treatments (*Fig 1B*). These changes are probably due to extractives being removed from the surface, thus exposing the polysaccharides and lignin underneath. Laccase also polymerizes lignans, which became adsorbed onto the surface.

The ESCA results showed that the fibril surface changed quite extensively during the enzymatic treatments, the largest change being the decrease in the surface coverage of extractives. The reason for this could be the large surface area of fibrils, which makes them a more accessible substrate for enzymes than the other fractions. At least carbohydrate-degrading enzymes, e.g. xylanase and endoglucanase, have been reported to act more aggressively towards fines than long fibers, and it has been suggested that enzymes might not act on the long fiber fraction at all if fines are present (Wong et al. 1997). Laccase treatment has also been found to be more effective in degrading the extractives present in white water than those found in TMP, since most of the white water extractives are either dispersed in the water phase or attached to the fines surfaces, where they can more easily react with laccase (Zhang et al. 2005).

Flakes

The reference treated flakes had a high surface coverage of lignin (*Fig 1C*), since they consist of pieces of middle lamella and outer cell wall layers together with broken or intact ray cells (Brecht and Klemm 1953; Mohlin 1977; Boutelje and Eriksson 1984). These are all rich in lignin and pectins and (Meier 1964; Hafren 1999). Ray cells also contain high amounts of xylan and extractives

Table 4. Normalized peak intensities taken from positive ToF-SIMS spectra of fibers, fibrils and flakes isolated from enzymatically treated pulps. LG = guaiacylic lignin units (peaks at 137 and 151 m/z), PS = polysaccharides (peaks at 115, 127, 133 and 145 m/z), FA = fatty acids (peaks at 239, 253, 257, 261, 263, 265, 267, 271, 279, 281, 283, 285, 295, 323, 351 and 369 m/z), RA = resin acids (peaks at 299, 300, 301, 302 and 303 m/z), TG = triglycerides (marker peaks at 313, 327, 335, 337, 339, 341, 551, 575, 595, 599, 600, 601, 602, 603 and 607 m/z) and ST+SE = sterols and steryl esters (peaks at 383, 397, 411 and 425 m/z).

Peak	LG	PS	FA	RA	TG	ST+SE
Fibers						
Reference	902	1277	368	102	135	124
Xylanase	1270	1270	549	120	389	193
Mannanase	1137	1417	495	104	363	193
Lipase	1419	1603	478	142	98	206
Laccase	462	1267	371	57	137	160
Pectinase	955	1345	508	103	490	207
Protease	873	1375	446	126	340	218
Fibrils						
Reference	507	1180	509	147	748	330
Xylanase	509	1156	463	73	896	284
Mannanase	490	1083	479	124	832	321
Lipase	537	1760	424	143	119	355
Laccase	365	1173	309	48	150	209
Pectinase	508	1297	484	153	795	345
Protease	489	1174	498	143	948	355
Flakes						
Reference	523	1121	363	56	448	274
Xylanase	539	1008	397	52	510	297
Mannanase	524	1218	305	46	338	220
Lipase	556	1813	373	62	107	453
Laccase	390	1247	313	41	149	229
Pectinase	474	1084	423	58	763	359
Protease	585	1425	411	67	228	287

(Hardell et al. 1980; Westermark and Capretti 1988; Karnis 1994) and around 20% of the reference treated flake surfaces were still covered with extractives (*Fig 1C*). The changes in the surface composition of flakes during the enzymatic treatments were small and mostly within the limits of experimental error. The surface coverage of extractives was increased by pectinase treatment and reduced by mannanase treatment. Pectinase treatment had similar effects on fibers and flakes, while on fibrils, pectinase treatment reduced the surface coverage of extractives. The reason for the increased surface coverage of extractives on flakes during pectinase treatment is probably readsorption of colloidal extractives from the water phase. In the mannanase treatment, some extractives may have been removed along with glucomannan.

Xylanase and lipase both reduced the coverage of lignin and increased that of polysaccharides on the surface of flakes. The xylanase treatment probably affected mostly ray cells, known to be rich in xylan, and while removing xylan from the surface, exposed cellulose. Some surface lignin was probably also removed along with xylan due to bonding between lignin and xylan, which have been suggested to exist (Sjöström 1993). The lipase treatment probably removed some extractives from the surface and thus revealed more polysaccharides on the surface. The ESCA results showed that less change took place on the surface of flakes than on the surface of fibrils during the enzymatic treatments.

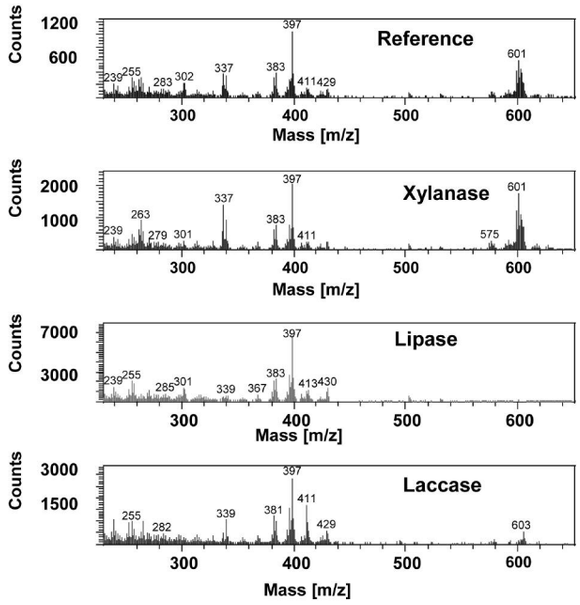

Fig 2. Parts of positive ToF-SIMS spectra of fibrils isolated from reference, xylanase, lipase and laccase treated TMPs.

Structural changes on the surface by ToF-SIMS

ToF-SIMS was used to detect changes in the chemical structures of the surface compounds brought about by the enzymatic treatments. ToF-SIMS gives the mass spectrum of a surface, as partly shown for fibrils from reference pulp and pulps treated with xylanase, lipase and laccase in *Fig 2*. From these positive ToF-SIMS spectra, it can already be seen that triglycerides (marked by a cluster of peaks around 600 m/z) disappeared from the surface of fibrils during lipase and laccase treatments. After laccase treatment, the peak at 411 m/z originating from steryl esters became quite intense. The normalized intensities of peaks originating from lignin, polysaccharides, fatty acids, resin acids, triglycerides, and sterols/steryl esters on fibers, fibrils and flakes are shown in *Table 4*. These values give an indication of the chemical changes taking place on the surfaces, but are by no means quantitative values. They can however be used to compare samples as done in the present paper.

Fibers

ToF-SIMS results show, that on the surface of fibers, the intensity of guaiacylic lignin peaks increased during xylanase, mannanase and lipase treatments, probably due to removal of glucomannan and xylan and extractives, respectively, thus exposing native lignin (*Table 4*). However, the removal of glucomannan and xylan could not be verified with ToF-SIMS, since the intensity of peaks originating from polysaccharides was increased and stayed unchanged in the mannanase and xylanase treatments, respectively, possibly due to exposure of cellulose.

The intensity of peaks originating from triglycerides decreased during lipase treatment, indicating their hydrolysis into fatty acids and glycerol. The fatty acids formed seemed to remain on the surface of fibers, and the increase of fatty acid content in the pulp filtrate was

caused by the hydrolysis of triglycerides in the filtrate. The intensity of peaks originating from polysaccharides increased due to removal of extractives. The increase in polysaccharide coverage was not observed with ESCA, indicating that this chemical change probably occurred on the very surface of the fibers. Another reason to this discrepancy may be that ToF-SIMS is more sensitive and has lower detection limits than ESCA.

The intensity of fatty acid peaks, triglyceride marker peaks as well as sterols/steryl ester peaks increased during mannanase, xylanase, pectinase and protease treatments (*Table 4*). The reason behind this could be destabilization of colloidal pitch and its adsorption onto fiber surface, which is known to occur during mannanase and xylanase treatments (Kantelinen et al. 1995). Laccase treatment lowered the intensity of resin acid and guaiacylic lignin peaks, probably due to oxidation of conjugated double bonds and phenolic compound, respectively.

Fibrils

According to ESCA results (*Fig 1B*), extractives were removed from the surfaces of fibrils in all the enzymatic treatments. However, ToF-SIMS spectra showed that triglycerides were removed selectively only by the lipase and laccase treatments and that laccase also reduced the intensity of fatty and resin acid peaks (*Table 4*). During the lipase treatment, triglycerides were hydrolyzed and thus their amount was reduced even on fibril surfaces. The formed fatty acids were dissolved together with a small part of the original fatty acids on the surfaces. At the same time, polysaccharides and some lignin was uncovered.

The oxidation of fatty and resin acids by laccase has been reported by Karlsson et al. (2001). In addition, the amount of sterols/steryl esters was reduced in the laccase treatment, probably by oxidation. Laccase treatment also reduced the amount of guaiacylic lignin units on fibril surfaces (*Table 4*), probably due to oxidation of the phenolic units (Kirk and Shimada 1985) in the surface lignin and/or redeposition of oxidized and polymerized lignans.

The effect of xylanase and mannanase treatments on the content of guaiacylic lignin, polysaccharides, fatty acids and sterols/steryl esters on fibril surfaces were small. However, both treatments increased the content of triglycerides, probably due to destabilization, followed by redeposition. Xylanase treatment also decreased the intensity of resin acid peaks. Pectinase and protease treatments also had a small effect on the chemistry of fibril surface. Only the content of triglycerides was increased, probably also due to redeposition onto the surface.

The reason for the conflicting results from ESCA and ToF-SIMS could lie in the different analysis depths of these techniques. ESCA analyzes all the extractives on the surface down to 5-10 nm, while ToF-SIMS analyzes only the outermost surface layers down to 1-2 nm. The surface coverage of extractives determined by ESCA decreases if the extractives on the surface either form a thinner layer on the surface or there are fewer or smaller patches of them. The thinner layer theory is supported by the results given by ToF-SIMS, since if there were fewer or smaller patches of extractives, the intensity of extractive peaks would decrease. But when the layer of extractives becomes thinner during enzymatic treatment, ToF-SIMS is not able to detect this change. In addition, it has to be taken into account that ESCA analysis can suffer from inadequate extraction of the samples. If extractives are retained on the surface after extraction, the calculation of surface coverage of both lignin and extractives, based on the averaged C-C percentages in high-resolution C 1s spectra before and after extraction, give erroneous results. ToF-SIMS, on the other hand, does not suffer from this kind of problem, since samples are analyzed as such.

Flakes

The intensity of triglyceride peaks in ToF-SIMS decreased on flakes during lipase, laccase, protease and mannanase treatments (*Table 4*). Lipase and laccase selectively reacted with triglycerides, but the effect of mannanase and protease was probably due to their removal along with other surface components.

Pectinase treatment increased the intensity of fatty acids, triglycerides, sterols and steryl esters probably because of destabilization and redeposition, as also shown by ESCA. Lipase treatment increased the relative intensity of sterol and steryl ester peaks, while removing triglycerides and fatty acids.

Laccase treatment reduced the intensity of peaks from guaiacylic units in lignin in ToF-SIMS analysis of flakes, as with the other fractions (*Table 4*), probably due to oxidation of phenolic lignin units. The intensity of polysaccharides increased slightly, possibly due to the removal of lignin and extractives. Although laccase treatment increased the intensity of the peak at 411 m/z originating from steryl esters notably, the content of sterols/steryl esters did not increase because of the decrease in the intensity of peaks originating from sterols at 383 and 397 m/z.

General comments

According to ToF-SIMS (*Table 4*), the amount of guaiacylic lignin units on the surfaces of the reference pulp components decreased in the order of fibers > flakes > fibrils. This supports our earlier finding (Kleen et al. 2003) that the fiber surface lignin is richer in guaiacylic units than e.g. the fibril surface lignin. The surface lignin on the flaky fines seems to contain about 40% less guaiacylic units than the lignin on the fibers (*Table 4*), though the lignin coverage is highest on flakes (*Fig 1*). These results strongly indicate different lignin structures on fiber surfaces compared to those on fibril and flake surfaces.

According to ToF-SIMS (*Table 4*), the amount of extractive signals on the surfaces of the reference pulp constituents decreased in the order of fibrils > flakes > fibers. This is in good agreement with the ESCA results (*Fig 1*).

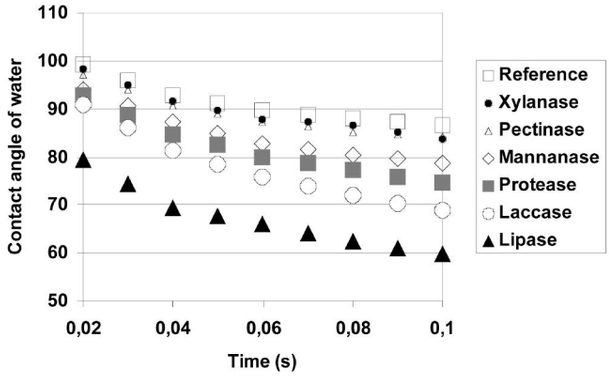

Fig 3. Contact angles of water on reference pulp and enzymatically treated pulp handsheets.

When comparing ToF-SIMS results of different pulp components, it has to be noted, that the surface roughness may influence the results. However, the peak intensities were always normalized to the total intensity of the spectrum in order to minimize the effect of different surface roughness of the samples.

The combined effect of enzymatic treatments and washing

The results discussed above showed that with enzymatic treatments, it is possible to remove extractives from the surfaces of pulp components, especially from fibrils. However, if the extractives are not removed from the water phase, they may redeposit onto the accessible surfaces, especially on fibrils. Therefore, the combined effect of enzymatic treatments and washing was studied by handsheet preparation and contact angle analysis.

The contact angles of water on handsheets prepared from enzymatically treated pulps as a function of time are shown in *Fig 3*. Contact angles are shown for a time period from 0.02 s to 0.1 s, since the drop volume was nearly constant during this time, indicating stable conditions during the measurements. The maximum deviation in the volume was 10% for the sheet made from lipase-treated pulp and less than 5% for the other samples.

All the enzymatic treatments followed by washing lowered the contact angles of pulp sheets, indicating an increase in hydrophilicity. The biggest decrease was induced by lipase treatment, which lowered the contact angle at 0.1 s by 30%. The reason for the increase in hydrophilicity was most probably the removal of extractives from the surface of the pulp components, since extractives are more hydrophobic than, say, polysaccharides.

Based on these results, lipase and laccase treatments followed by washing could be used to remove extractives from the surface and make the pulp more hydrophilic.

Conclusions

Fibrils were the most reactive fraction of TMP in the extensive treatments with commercial enzyme preparations, as analyzed by ESCA. All the enzymes studied here – xylanase, mannanase, lipase, pectinase, laccase and protease – reduced the coverage of extractives on the surface of fibrils, while the effect was less obvious in the case of fibers and flakes. According to the ToF-SIMS results, the intensity of extractives was still as high on the outermost surface of fibrils as on other fractions. It is therefore suggested that the surface layer of extractives on fibrils became thinner due to enzymatic treatments and that this change was detected by ESCA. On the other hand, ToF-SIMS could analyze the film of extractives on the surface, regardless of its thickness.

All six enzymatic treatments followed by washing lowered the contact angle of water on pulp handsheets, most probably due to removal of extractives. Lipase and laccase removed extractives effectively from the surfaces of pulp components as shown by ESCA, and lipase reduced the contact angle the most, followed by laccase. Lipase and laccase treatments combined with washing could then be used to selectively remove extractives from the pulp surface and make the pulp more hydrophilic.

The results from ESCA and ToF-SIMS gave complementary information about the chemistry and arrangement of chemical structures on the surface of pulp components. ESCA made it possible to study how each enzymatic treatment affected the content of surface components. With ToF-SIMS, it was possible to identify the different extractives components and to study their behavior during treatment. It became evident that lipase and laccase were the enzyme preparations that selectively reacted with extractives. Other enzymes may have decreased or increased the extractives coverage on the surface, but this was due to changes in the stability of the system and the removal or redeposition of extractives. Laccase also selectively oxidized phenolic lignin structures, which was seen as decrease in the intensity of peaks from guaiacylic units in lignin on the surfaces of pulp components. ToF-SIMS was thus found to be well suited for studying the effect of enzymatic treatments on the surface compounds of pulp components.

When ESCA and ToF-SIMS are used for surface analysis, the differences in their analysis depth and sensitivity have to be taken into account. In addition, ToF-SIMS is not a quantitative analysis method for organics, while ESCA results can be influenced by inadequate extraction.

Acknowledgements

We thank Drs Leena-Sisko Johansson and Joseph Campbell for performing the ESCA analyses. Financial support from the Finnish Cultural Foundation, the National Technology Agency of Finland (TEKES), The Walter Ahlström Foundation and the Foundation of Technology (TES) is gratefully acknowledged.

Literature

ISO standard 5263 (1995): Pulps – Laboratory wet disintegration – Part 3: Disintegration of mechanical pulps at >= 85 degrees C.

SFS-EN ISO standard 5269-1 (2000): Pulps – Preparation of laboratory sheets for physical testing – Part 1: Conventional sheet-former method.

Barsberg, S., Thygesen, L.G. and Felby, C. (1998): Detection of laccase induced modification of beech and spruce TMP fibres by luminescence spectroscopy, 7th Int. Conf. on Biotechnology in the Pulp and Paper Industry, Vancouver, BC, Canada, June 16-19, 1998, CPPA, Montreal, Canada, pp. C123-126.

Buchert, J., Mustranta, A. and Holmbom, B. (2002): Enzymatic control of dis-

solved and colloidal substances during mechanical pulping, In: Viikari, L. and Lantto, R. (eds.), Biotechnology in the pulp and paper industry, Progress in biotechnology, Elsevier, Amsterdam, pp. 271-280.

Buchert, J., Mustranta, A. Spetz, P., Ekman, R. and Luukko, K. (1999): Use of enzymes for modification of dissolved and colloidal substances in process waters of mechanical pulping, Pre-Symposium of the 10th Int. Symp. on Wood and Pulping Chemistry (ISWPC), Seoul, Korea, June 2-4, 1999, Tappi Korea, pp. 115-119.

Buchert, J., Siika-aho, M., Bailey, M., Puls, J., Valkeajärvi, A., Pere, J. and Viikari, L. (1993): Quantitative determination of wood derived soluble oligosaccharides by HPLC, Biotechnol. Tech. 7(11), 785-790.

Börås, L. and Gatenholm, P. (1999): Surface properties of mechanical pulps prepared under various sulfonation conditions and preheating time, Holzforschung, 53(4), 429-434.

Dorris, G. M. and Gray, D.G. (1978): The surface analysis of paper and wood fibres by ESCA. II. Surface composition of mechanical pulps, Cellulose Chem. Technol. 12(6), 721-734.

Ekman, R., Eckerman, C. and Holmbom, B. (1990): Studies on the behaviour of extractives on the mechanical pulp suspensions, Nord. Pulp Paper Res. J. 5(2), 96-103.

Fardim, P. and Holmbom, B. (2005): Origin and surface distribution of anionic groups in different papermaking fibres, Colloids Surf. A 252, 237-242.

Hafren, J. (1999): Ultrastructure of wood cell wall, Ph.D. Thesis, Royal Institute of Technology, KTH, Stockholm, Sweden.

Hardell, H.-L., Leary, G.J., Stoll, M. and Westermark, U. (1980): Variations in lignin structure in defined morphological parts of spruce, Svensk Papperstidning 83(4), 44.

Hata, K., Matsukura, M., Taneda, H. and Fujita, Y. (1996): Mill-scale application of enzymatic pitch control during paper production, In: Jeffries, T.W. and Viikari, L. (eds.), Enzymes for pulp and paper processing, American Chemical Society (ACS), Washington DC, pp. 280-296.

Kangas, H. and Kleen, M. (2004): Surface chemical and morphological properties of mechanical pulp fines, Nord. Pulp Paper Res. J. 19(2), 191-199.

Kangas, H., Pöhler, T., Heikkurinen, A. and Kleen, M. (2004): Development of the mechanical pulp fibre surface as a function of refining energy, J. Pulp Paper Sci. 30(11), 298-306.

Kantelinen, A., Jokinen, O. Sarkki, M.-L., Pettersson, C., Sundberg, K., Eckerman, C., Ekman, R., and Holmbom, B. (1995): Effect of enzymes on the stability of colloidal pitch, 8th Int. Symp. on Wood and Pulping Chemistry (ISWPC), Helsinki, Finland, June 6-9, 1995, KCL, Jyväskylä, Finland, pp. 605-612.

Karlsson, S., Holmbom, B., Spetz, P., Mustranta, A. and Buchert, J. (2001): Reactivity of Trametes laccases with fatty and resin acids, J. Appl. Microbiol. Biotechnol. 55, 317-320.

Karnis, A. (1994): The mechanisms of fibre development in mechanical pulping, J. Pulp Paper Sci. 20(10), J280.

Kirk,T.K. and Shimada, M. (1985): Lignin biodegradation: The microorganisms involved and the physiology and biochemistry of degradation by white-rot fungi, In: Higuchi, T. (ed), Biosynthesis and biodegradation of wood components, Academic Press Inc., Orlando, pp. 579.

Kleen, M. (2000): ToF-SIMS as a new analytical tool for studies of pulp fiber surface chemistry, International Symposium on cellulose and lignocellulosics chemistry 2000 (ISCLC 2000), Kunming, China, Dec. 16-18, 2000, pp. 290-294.

Kleen, M., Kangas, H. and Laine, C. (2003): Chemical characterization of mechanical pulp fines and fiber surface layers, Nord. Pulp Paper Res. J. 18(4), 361.

Kleen, M., Sjöberg, J. Dahlman, O., Johansson, L.-S., Koljonen, K. and Stenius, P. (2002): The effect of ECF and TCF bleaching on the chemical composition of soda-anthraquinone and kraft pulp surfaces, Nord. Pulp Paper Res. J. 17(3), 357-363.

Kokkonen, P., Fardim, P. and Holmbom, B. (2004): Surface distribution of extractives on TMP handsheets analyzed by ESCA, ATR-IR, ToF-SIMS and ESEM, Nord. Pulp Paper Res. J. 19(3), 318-324.

Koljonen, K., Stenius, P. and Buchert, J. (1997): The surface chemistry of PGW pulp fibre fractions, Int. Mechanical Pulping Conf. (IMPC), Stockholm, Sweden, June 9-13, 1997, SPCI, Stockholm, Sweden, pp. 407-411.

Luukko, K., Kemppainen-Kajola, P. and Paulapuro, H. (1997): Characterization of mechanical pulp fines by image analysis, Appita J. 50(5), 387.

Luukko, K., Laine, J. and Pere, J. (1999): Chemical characterization of different mechanical pulp fines, Appita J. 52(3), 126-131.

Meier, H. (1964): General chemistry of cell walls and distribution of the chemical constituents across the walls, In: Zimmerman, M.H. (ed.), The formation of forest trees, Academic Press, NewYork, pp. 137-151.

Mosbye, J. (2003): Colloidal wood resin. Analysis and interactions, Ph.D Thesis, Norwegian University of Science and Technology, Trondheim, Norway.

Mustranta, A., Buchert, J., Spetz, P. and Holmbom, B. (2001): Treatment of mechanical pulp and process waters with lipase, Nord. Pulp Paper Res. J. 16(2), 125-129.

Mustranta, A., Koljonen, K. Pere, J., Tenkanen, M., Stenius, P. and Buchert, J. (2000): Characterization of mechanical pulp fibres with enzymatic, chemical and immunochemical methods, 6th Europ. Workshop on Lignocellulosics and Pulp (EWLP), Bordeaux, France, September 3-6, 2000, ATIP, Paris, France, pp. 15-18.

Niku-Paavola, M.-L., Karhunen, E., Salola, P. and Raunio, V. (1988): Lignolytic enzymes of the white-rot fungus Phlebia radiata, Biochem. J. 254, 877-884.

Peng, F., Ferritsius, R. and Ängsås, U. (2003): Mechanical pulping with pectinase pretreatment of wood chips, Int. Mechanical Pulping Conf. (IMPC), Quebec, Canada, June 2-5. 2003, PAPTAC, pp. 335-340.

Poppius-Levlin, K., Wang, K., Tamminen, T., Hortling, B., Viikari, L. and Niku-Paavola, M.-L. (1999): Effects of laccase/HBT treatment on pulp and lignin structure, J. Pulp Paper Sci. 25(3), 90.

Reid, I. and Ricard, M. (2000): Pectinase in papermaking: solving retention problems in mechanical pulps bleached with hydrogen peroxide, Enzyme Microbial Technol. 26, 115.

Sjöström, E. (1993): Wood chemistry. Fundamentals and applications. 2. Ed., Academic Press, San Diego, p. 293.

Thornton, J. (1994): Enzymatic degradation of polygalacturonic acids released from mechanical pulp during peroxide bleaching, Tappi J. 77(3), 161.

Thornton, J., Eckerman, C., Ekman, R. and Holmbom, B. (1996): Treatment of alkaline bleached mechanical pulp with pectinase, U.S. Patent 5,487,812.

Westermark, U. (1999): The content of lignin on pulp fiber surfaces, 10th Int. Symp. on Wood and Pulping Chemistry (ISWPC), Yokohama, Japan, June 7-10, 1999, Japan TAPPI, pp. 40-42.

Westermark, U. and Capretti, G. (1988): Influence of ray cells on the bleachability and properties of CTMP and kraft pulps, Nord Pulp Paper Res. J. 3(2), 95-99.

Wong, K.K.Y., Richardson, J.D., Anderson, K.B. and Clark, T.A. (1997): Hydrolysis and modification of mechanical pulp using carbohydrate-degrading enzymes. 9th Int. Symp. on Wood and Pulping Chemistry (ISWPC), Montreal, Que, Canada, June 9-12, 1997, CPPA, Montreal, Canada, pp. 121-1-121-5.

Wood, J.R., Grondin, M. and Karnis, A. (1991): Characterization of mechanical pulp fines with a small hydrocyclone. Part I: The principle and nature of the separation, J. Pulp Paper Sci. 17(1), J1.

Zhang, X., Renaud, S. and Paice, M. (2005): The potential of laccase to remove extractives present in pulp and white water from TMP newsprint mills, J. Pulp Paper Sci. 31(4), 175.

Zhang, X., Stebbing, D.W., Saddler, J.N., Beatson, R.P. and Kruus, K. (2000): Enzyme treatments of the dissolved and colloidal substances present in mill white water and the effects on the resulting paper properties, J. Wood Chem. Technol. 20(3), 321.

Örså, F. and Holmbom, B. (1994): A convenient method for the determination of wood extractives in papermaking process waters and effluents, J. Pulp Paper Sci. 20(12), J361.

Manuscript received May 18, 2007
Accepted July 18, 2007